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Thematic Review

Microorganisms: the secret agents of the biosphere, and their key roles in biotechnology

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Abstract: We present a survey of projects that have been funded by FAPESP under the BIOTA-Microorganisms program. These projects generated a wide variety of results, including the identification of novel antibacterial-producing microorganisms, the characterization of novel microbial enzymes for industrial applications, taxonomic classification of novel microorganisms in several environments, investigation of the soil and mangrove microbial ecosystems and its influence on endangered plant species, and the sequencing of novel metagenome-assembled genomes. The results surveyed demonstrate the importance of microorganisms in environments that play important roles in human activities as well as the potential that many of these microorganisms have in contributing to biotechnological applications crucial for human survival in the 21st century.

Keywords: *Microbial diversity; bioprospecting; enzymes; secondary metabolites; metagenomics; microbiome.*

Micro-organismos: os agentes secretos da biosfera, e seus papéis determinantes na biotecnologia

Resumo: Apresentamos um levantamento comentado de projetos financiados pelo programa BIOTA-Micro-organismos. Estes projetos geraram uma variada gama de resultados, incluindo a identificação de novos micro-organismos produtores de compostos antibacterianos, a caracterização de novas enzimas microbianas para usos industriais, classificação taxonômica de novos micro-organismos presentes em diversos ambientes, investigação de ecossistemas microbianos em solos e mangues e sua influência sobre plantas ameaçadas, e o sequenciamento de vários novos genomas microbianos derivados de metagenomas. Os resultados descritos demonstram o papel-chave de micro-organismos em ecossistemas importantes para atividades humanas, assim como o potencial que vários desses micro-organismos tem de contribuir para aplicações biotecnológicas cruciais para a sobrevivência humana no século 21.

Palavras-chave: *Diversidade microbiana; bioprospecção; enzimas; metabólitos secundários; metagenômica; microbioma.*

Introduction

Microorganisms (archaea, bacteria, viruses, and eukaryotic microorganisms such as protozoans, fungi, and algae) are widespread and ubiquitous in the biosphere. In the case of archaea and bacteria, Whitman, Coleman & Wiebe (Whitman, Coleman & Wiebe 1998), estimated that their total carbon is 60–100% of the estimated total carbon in plants. This means that microorganisms play key roles in nearly all large-scale processes on Earth. Knowing their diversity and roles is a primary research objective in environmental science, but also in many other areas, such as agriculture and forestry.

In many cases microbes are part of an interconnected community of host cells and associated microbiota, with an intrinsic interdependent relationship. Microorganisms therefore are key to the maintenance of homeostasis of all living organisms and ecosystems (Paasch & He 2021).

Brazil is a country bestowed with a rich biodiversity, ranking at the top of the 17 most diverse countries on Earth. There are many different biomes throughout the national territory, the main ones being the Amazon, Cerrado, Caatinga, Pantanal, Atlantic Forest, Pampa or Southern Fields and the Coastal biome. Within these biomes there is an extensive community of microorganisms yet to be characterized, catalogued, and explored, whether present in the air, water, soil or associated with other living organisms. In plants, they are called endophytic when they live the whole or part of their life cycle within the host's organs and epiphytic when found on the surface, such as leaves. Unfortunately, the habitats in which they are found are nowadays devastated by fires, plantations, cattle pastures, and pollutant contamination, due to the lack or non-implementation of environmental preservation policies by the Brazilian government, which jeopardizes this unexplored niche.

Currently, knowledge of a microbial community is not limited to identifying isolated organisms recovered from environmental samples or associated with a particular host, but also how this diverse population of microbes communicates with each other and with their environment. It is a fact that the advent of molecular techniques provided a great advance in the knowledge of the genetic and taxonomic diversity of microorganisms in environmental samples, since such techniques enable the direct analysis of microbial diversity, without the need for cultivation, allowing, for example, the phylogenetic identification of cultures based on sequencing of the 16S rRNA gene (Andreote & Pereira 2017).

Tools used for microbial identification, such as metagenomics, have been associated with others for metabolic analysis, such as metabolomics. This joining of different data is possible due to the improvement on bioinformatics that allows to cross-reference information. This coalescence of techniques is the new trend established for this decade in microbial research and great advances are expected to expand our knowledge in this field, besides refining and improving diverse approaches that make it possible to study microorganisms that are not so easy to grow in laboratories and to monitor even more closely how the communication between plant/microorganism takes place.

Microbes offer solutions to human needs since ancient times, with bread, beer and wine fabrication using yeast. Throughout the years, microbial uses expanded to many areas, providing products and services to a diverse number of applications, in what is commonly called Microbial Biotechnology. Their genomic versatility, metabolic richness, ubiquity, and the possibility to large-scale fermentation to supply industrial uses put microbes as special players in the achievement of the United Nations Sustainable Development Goals (SDGs) to a healthy and fair planet by 2030 (Timmis et al. 2017) (Figure 1). Nowadays, the applications include disease prevention and diagnostics, drug development, food and nutritional supplement industries, energy and alternative fuels, environmental monitoring, and waste management, among others.

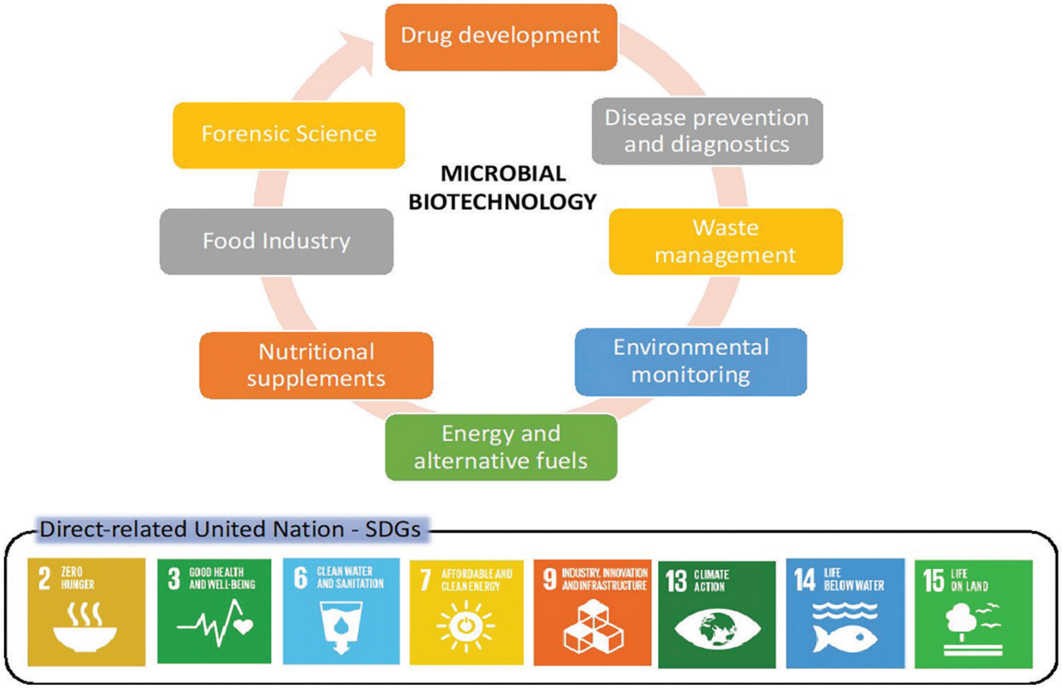

Figure 1. Applications of Microbial Biotechnology and its relation to the United Nations Sustainable Development Goals (SDGs).

This work summarizes scientific contributions by research groups that have been funded by FAPESP within the BIOTA/FAPESP – Microorganisms Program. The purpose of such investigations included identification of novel antibacterial producing microorganisms, characterization of novel enzymes for industrial applications, taxonomic classification of novel microorganisms in several environments and investigation of the soil microbial ecosystem and its influence on endangered plant species.

In the text that follows, we refer to FAPESP-funded projects by the BIOTA/FAPESP – Microorganisms program by their titles and grant numbers.

Microbial Diversity and Taxonomy

The diversity of microorganisms in environmental samples has been the focus of several investigative efforts supported by the BIOTA/FAPESP Program. These organisms include prokaryotes, a group that encompasses organisms from the phylogenetically unrelated domains Bacteria and Archaea, and eukaryotes, such as fungi and protozoa, as well as viruses.

The identification of microorganisms is hampered by the difficulty of cultivating in the lab the vast majority of microbes. On the other hand, the availability of cultivation-independent methods (i.e., metagenomics) associated with DNA/protein sequence-based phylogenetic classification has helped increase our knowledge on the diversity of existing microorganisms. The BIOTA/FAPESP initiative has supported projects that aimed to identify novel microorganisms using both cultivation and cultivation-independent methods.

The proposal entitled "Biodiversity and taxonomy of protozoofauna present in samples of raw surface water and sediment from the Atibaia River, in the city of Campinas, São Paulo, Brazil" (grant 2011/50244-8) aimed at the characterization of the biodiversity of ciliated protozoa in both water and sediment of the Atibaia River, applying both molecular and conventional taxonomic identification. A similar project entitled "Diversity of free-life amoebas in the Monjolinho river in the state of São Paulo – morphological and molecular approaches" (grant 2018/20693-4) had the objective of identifying amoebas, both pathogenic and non-pathogenic, in different positions along a river that crosses the city of São Carlos.

The first project, coordinated by Regina Maura Bueno Franco, identified a total of 66 taxa belonging to 55 ciliate genera (Bonatti & Franco 2016). The survey of ciliated protozoa is of great impact, as these organisms are bioindicators of water quality and there is a lack of research about their diversity, resulting in their exclusion from environmental management programs and biodiversity conservation. Amoebas are another class of protozoa that can live both as free organisms in water sources and as human pathogens, causing serious and therefore fatal diseases. The second project, coordinated by Otavio Henrique Thiemann, has similarly surveyed the water of a river in the state of São Paulo. In this survey, amoebas of the genera *Acanthamoeba* and *Naegleria* were identified, representing a potential risk for the population. In total, amoebas of five different genera were identified and the methodologies for sample collection and both morphological and molecular classifications were established (Bellini et al. 2020). The survey of ciliated and amoeba protozoa in the Atibaia and Monjolinho rivers extended the knowledge and the occurrence of these protozoa in Brazilian lotic systems.

Going beyond the traditional environmental sampling sites, as water streams and soil, the microbial diversity has been explored in other diverse niches under the BIOTA/FAPESP Program. The project conducted by Fernando Luis Consoli, "Diversity, ecology and biotechnological potential of the symbiont bacteriofauna associated with insects" (grant 2011/50877-0) investigated the association between bacteria and insects and its key roles in the host defense, nutrition, and reproduction. Surveying the Actinobacteria diversity in the midgut of stinkbugs (Hemiptera: Pentatomidae) species resulted in the identification of 34 phylotypes distributed in 11 different actinobacteria families (Scopel & Consoli 2018). This work indicated that stinkbugs harbor a diverse collection of actinobacterial species. Although actinobacteria are known as a source of biomolecules, the ecological features of these symbionts in the stinkbug biology remain unknown and is the focus of additional research.

As the above-mentioned successful projects funded by the BIOTA/FAPESP program demonstrate, the investigation of microbial diversity is not limited to cataloguing species in specific environments. The microbial identification and taxonomic classification of species has resulted in direct and practical results for human public health, agriculture, soil conservation, endangered plant species preservation efforts, among others.

Bioprospecting the Biotechnological Potential of Microorganisms

Projects in the microbial biotechnology field funded by the BIOTA/FAPESP program assessed the environmental microbial communities (not including human microbiota), including those microorganisms associated to different biological matrices (plants, insects, marine organisms, etc.) and sediments and water from different Brazilian biomes, with different applications. The most explored applications included the isolation of bioactive compounds with pharmaceutical potential, the use of environmental-related strains and isolated enzymes in biocatalysis, expanding the natural product chemical space, and the recovery of strains with impact in crop productivity and resistance. Figure 2 shows that among the 46 projects funded by BIOTA/FAPESP program on microbiology, at least 29 involved a biotechnological application. In the following paragraphs, we will discuss some results of the supported projects to illustrate the impact of the BIOTA/FAPESP program on each of these fields.

A previous review published in 2014 by Roberto Berlinck and collaborators (Ioca et al. 2014) discussed the research on microbial natural products in Brazil. According to the authors, until 2013, 84% of the studies on microbial secondary metabolites assessed fungal strains, and most of these strains were isolated from plants. Studies on endophytic fungi increased worldwide, prompted by the information that some potent anticancer compounds previously isolated from plants, as paclitaxel, camptothecin, podophyllotoxin and vincristine, among others, were, in fact, produced by plant-associated microbes (Ioca et al. 2014, Newman & Cragg 2020). Nonetheless, (Ioca et al. 2014) emphasized that the number of projects dealing with microbial diversity and biodiscovery funded by the BIOTA/FAPESP program was still modest in 2013. Unfortunately, this did not change in the past 10 years, and we will need much more enterprises and funding initiatives for a proper exploration of our microbial diversity.

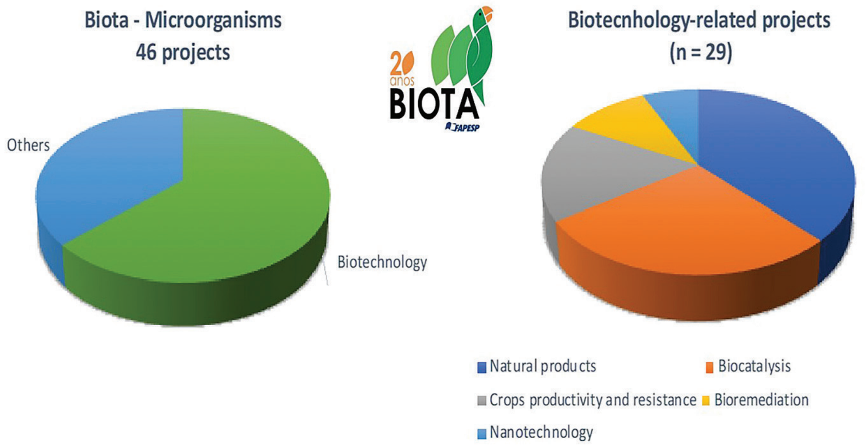

Figure 2. Distribution of the projects supported by BIOTA/FAPESP program along the different areas of research. A – From a total of 46 funded projects, 29 had biotechnological goals. B – Distribution by biotechnological application.

Marine microorganisms have growing importance in the search for microbial natural products. Two projects were funded by FAPESP focusing on marine microorganisms collected along the Brazilian coast and islands ("Biodiversity components, and their metabolic characters, of Brazilian Islands", grant 2013/50228-8, and "Integrative approach on the sustainable prospecting of marine natural products: from diversity to anticancer compounds", grant 2015/17177-6). The first project coordinated by Roberto Berlinck (grant 2013/50228-8) focused mainly on metabolites produced by microorganisms (fungi and bacteria) isolated from marine invertebrates, as sponges and ascidians, with activity against neglected diseases, including malaria, Chagas disease and leishmaniosis. This initiative led to the isolation of the marine bacterium *Pseudovibrio denitrificans* Ab134 from the Brazilian endemic sponge *Arenosclera brasiliensis*, that was able to produce bromotyrosine-derived alkaloids previously isolated from sponges with antiparasitic activity (Nicacio et al. 2017). The project coordinated by Leticia Costa-Lotufo (grant 2015/17177-6) focused on the study of anticancer compounds from marine bacteria using a target-oriented screening. This innovative strategy led to the isolation of compounds with unique targets, such as chromomycin A5 that directly binds to TBX2 transcription factor, inhibiting cancer cell proliferation (Sahm et al. 2020). Indeed, these two projects led to the isolation of thousands of bacterial and fungal strains, that can be further used with multiple purposes in microbial biotechnology. See also Costa-Lotufo et al. (in press), regarding bioprospecting macroalgae, marine and terrestrial invertebrates and their associated microbiota in the BIOTA-FAPESP Program.

Bioprospecting efforts were also expanded to other biological matrices as insects. In a project led by Monica T. Pupo (grant 2011/50869-8), a better understanding of the role of natural products in the interactions between microbial symbionts and their social insect hosts was gained, and new compounds with therapeutic potential were discovered. This work was based on hundreds of samples of social insect colonies obtained in Amazonia, Atlantic Rain Forest and Cerrado environments, including fungus-cultivating ants and stingless bees (Paludo et al. 2018, Silva-Junior et al. 2018, Ortega et al. 2019, Menegatti et al. 2020). In addition, in an ICBG collaborative project ("Novel therapeutic agents from the bacterial symbionts of Brazilian invertebrates", grant 2013/50954-0) between M. Pupo's Lab at the School of Pharmaceutical Sciences in Ribeirão Preto, University of São Paulo, and Prof. Clardy's Group at Harvard Medical School, a new polyketide, cyphomycn, was described from a *Streptomyces* sp. strain associated to Cyphomyrmex ants with potent fungicide properties against ant and human fungal pathogens (Chevrette et al. 2019, Bugni et al. 2020).

Biocatalysis involves the use of living organisms (fungi, bacteria, plants, and algae) to perform the chemical transformation of organic compounds, being a very important strategy to obtain pharmaceuticals using greener manufacturing processes (Aldridge 2013). According to (Birolli et al. 2015), in Brazil there is still a reduced number of research groups applying biocatalysis to transform organic compounds, and most of the research is based on the use of isolated lipases and yeast to perform enzymatic reactions. A lipase produced by the endophytic fungus *Cercospora kikuchii* was studied under the project "Bioprospecting in fungi: the search of lead compounds for drug design and enzymes for pharmaceutical and industrial applications" (Costa-Silva et al. 2014) (grant 2004/07935-6). Although many projects aimed at the isolation and characterization of lipases, other enzymes with potential industrial applications were also isolated and characterized, including tannases, phytases, cellulases, among others (Sato et al. 2016, Alves et al. 2018, Cavalcanti et al. 2018). The use of environmental fungal strains in the biotransformation of organic molecules is also an important addressed goal (de Carvalho et al. 2010, Capel et al. 2011, Severiano et al. 2013, de Souza et al. 2021).

These are just a few examples of the biotechnological potential of the microbial diversity. Nonetheless, it is already possible to observe the impact of the generated basic knowledge on the number of projects funded by FAPESP as Innovative Research in Small Business (PIPE) and University-Industry Cooperative Research (PITE). These projects aim at the development of innovative solutions and entrepreneurship initiatives in a myriad of applications, including optimizing seedling production, development of microbial-based bioproducts and remediation strategies for the recovery of areas impacted by contaminants.

Microorganisms Associated with Plants and Agricultural Systems

In plant-microbe associations, plants respond to microorganisms by activation of different gene pathways that can be limited to the site of infection and/or may lead to a systemic resistance by plant organs that are more distantly located. In the acquisition of plant resistance, the produced enzymes, and metabolites, play a central role in the defense against pathogens. Microbial interactions can also be parasitic, when one organism benefits at the expense of another; mutualistic, in such a way that both organisms benefit from each other; or commensal, in which one organism benefits at no cost or benefits to the other (Phelan et al. 2011, Wright et al. 2013).

Between 1962 and 1991, the main objectives among the projects submitted to FAPESP for research funding focused on the cultivation of microorganisms, essentially for biotechnological applications and pollution indicators. In the following decades, and mainly due to the creation of the BIOTA/FAPESP program, the objectives were extended towards the understanding of the microbial community and its interactions, especially those occurring in the soil as well as in the rhizosphere and plant/bacteria associations.

Actinobacteria are a taxonomically wide and metabolically diverse group of bacteria. In several ecological niches the knowledge on the diversity and functional roles of these microorganisms is scarce. With the pressing need for the development of novel antibiotics for use in clinical treatments of antibiotic resistant infections it is worrisome that about 50% of known antibiotics are produced by actinomycetes and of these a single genus, *Streptomyces*, is responsible for 90%, although other genera are known to produce unique classes of antibiotics. Consequently, due to their economic and biotechnological potential, the investigation of the diversity of actinomycetes has an urgent appeal. A project coordinated by Tiago Domingues Zucchi entitled “Diversity and polyphasic taxonomy of actinobacteria isolated from reforestation areas” (grant 2011/14333-6) was focused on the identification of actinobacterial species in restored areas and on the determination of the soil attributes related to the bacterial community profile in each site. The researchers demonstrated that the distribution of bacteria is correlated to soil chemical characteristics and to the plant diversity during the rainforest restoration process.

In a related project coordinated by Elke Cardoso, “Plant biodiversity and edaphic organisms in natural and impacted *Araucaria angustifolia* ecosystems in the state of São Paulo” (grant 2001/05146-6), the soil microbial diversity of different Araucaria forests was evaluated, with special attention to rhizobacteria, mycorrhizal fungi and diazotrophic bacteria. This interdisciplinary study demonstrated the close connection between plant species and the soil microbiota, contributing to the conservation effort of *Araucaria angustifolia,* which is an endangered tree species.

Endophytic microorganisms, i.e., organisms that live within the plant tissue in a symbiotic association, were further investigated in the project “Diversity of endophytic microorganisms and their biotechnological potential” (grant 1999/09177-1), coordinated by Itamar Soares de Melo. The project identified endophytic fungal and bacterial species, such as *Escherichia fergusonii*, *Acinetobacter calcoaceticus* and *Salmonella enterica*, which were shown to increase coffee plant growth. On the other hand, the species *Brevibacillus choshinensis*, *S. enterica*, *Pectobacterium carotovorum*, *Bacillus megaterium*, *Microbacterium testaceum* and *Cedecea davisae* were shown to significantly reduce rust severity on coffee leaf discs.

Opuntia ficus-indica, a cactus found in the dry regions of Brazil, has been introduced to other Brazilian environments in order to support animal and human nutrition during droughts. The project coordinated by Itamar Soares de Melo identified 68 bacterial strains associated with this plant, among them *Agrobacterium radiobacter*, *Klebsiella trevisanii*, *Enterobacter agglomerans* and *Paracoccus denitrificans*, which were found to contribute to the increase of root dry matter.

The studies mentioned above revealed the potential of increasing plant production and soil conservation by managing the soil and/or the plant-associated microbiota.

The rhizosphere is a region of high biological activity due to substances released by the roots, which provide an ideal environment for the establishment of a microbial community. In this scope, the knowledge of the genetic and functional diversity of the microbiota associated with the rhizosphere is of fundamental importance for the advancement and understanding of the existing relationships between microorganisms and plants. The project “Rhizosphere of *Senna spectabilis*: studies of plant/microorganism interactions using metabolomic tools” (grant 2011/50816-1) aimed to understand the molecular interactions in the rhizosphere of *Senna spectabilis* (Fabaceae), using a rational approach to study the relationship between this interesting niche and the microorganisms that inhabit it, making it a rich potential source of novel bioactive metabolites. In this project, a collection of microorganisms was obtained, including filamentous fungi and bacteria, which opened a new field of research at the Nuclei of Bioassays, Biosynthesis and Ecophysiology of Natural Products Center (NuBBE). The study of the specimens resulted in the discovery of an array of molecules and allowed the establishment of metabolomic protocols that guided further decisions of the researchers regarding metabolite purification. An in-house database was created hosting spectroscopic and spectrometric data (MS and ^{1}H, ^{13}C NMR) of the isolated molecules from diverse microorganisms, nowadays integrated into a much larger database called NuBBEDB (Pilon et al. 2017) (https://nubbe.iq.unesp.br/portal/nubb). The formulation and conception of algorithms to analyze highly complex mixtures using NMR5-7 (Selegato et al. 2019, Valli et al. 2019, Valli et al. 2019) allowed the deconvolution and dereplication of spectra from OSMAC (One Strain, Many Compounds) cultures. As a result of these efforts, a patent was deposited through the Patent Cooperation Treaty (PCT) (Freire & Castro-Gamboa 2015) describing the algorithms and their functionality towards the dereplication of NMR spectra sets. The dereplication of mass spectrometry data has emerged as an important strategy for the structural elucidation of known compounds and allowed the identification of metabolites present in the extracts of some rhizosphere fungi, such as *Fusarium solani* and *F. oxysporum*. By comparison with consolidated databases and using molecular networking, a complete view of metabolic production and the discriminant analysis of chemical profiles were achieved, allowing the identification of several metabolites that discriminated both cultures (Vieira et al. 2020).

Microbial strains with impact in crop productivity and resistance have also been focus of research supported by BIOTA/FAPESP. Studies with microorganisms isolated from coffee tissues (including 217 strains of endophytic bacteria and 17 fungi) showed that while some of the bacterial strains were able to reduce the severity of coffee leaf rust (*Hemileia vastatrix*) and increase plant growth, none of the endophytic fungal strains induced plant growth or reduced coffee leaf rust (Silva et al. 2012). More recently, the project “Biodiversity of *Trichoderma* spp. from the Amazon Forest and bioprospecting of hydrolytic enzymes and bioactive compounds” (grant 2013/26659-9) found phosphorus-solubilizing *Trichoderma* spp. with a positive impact on soybean growth, reaching 41% positive response, emphasizing the role of these microorganisms as promising biofertilizer agents (Bononi et al. 2020).

Ongoing research exploring plant-associated microbes will allow us to understand more of the chemical complexity of this association, their chemical communication, and their impact on the production of metabolites with diverse practical applications.

Microbes and Biomass Transformation

The project entitled "Bioprospecting filamentous fungi aiming at the application of holoenzymes in biorefinery" (grant 2010/52322-3), coordinated by Maria de Lourdes Polizeli, aimed to search for filamentous fungi as genetic and biochemical resources for biotechnological purposes, with the perspective of sustainable development of the biofuel second-generation ethanol. Unlike the so-called "first generation ethanol" obtained from sucrose, it is generated from straw, sugarcane bagasse, or other agricultural residues, which are currently underused.

Brazil is an agricultural country, and the amount of agro-industrial waste generated exceeds consumption limits and leads to environmental contamination. In addition to sugarcane bagasse, seasonal production residues can be included in this scenario, such as coconut fibers, corn bran, cob, peanut, rice and citrus husks, soybean bran, wheat bran, cassava, among others. All residues are made up of substantial amounts of cellulose, hemicellulose, and lignin. These polymers are intrinsically associated, forming a complex architecture of the cell wall and allowing limited access to the action of holoenzymes. As a result, the ease of obtaining biocatalysts released into the medium, high activity of biomass degradation, low inhibition by the products formed, and enzymatic thermostability are fundamental characteristics for the success of a saccharification process. These characteristics are obtained with enzymes of fungal origin since these organisms have a high capacity for cellular secretion.

In this context, the project aimed to form a "National Network of Filamentous Fungi", isolating fungi from the six national biomes, with a majority production of cellulases, xylanases, and ligninases. Filamentous fungi from the Amazon region, Cerrado, Atlantic Forest, Pantanal, Caatinga, and Pampas were isolated, but samples were also collected from the Iguaçu National Park and the Brazilian seacoast.

The project reconciled bioprospecting studies of filamentous fungi, the environment, and new government policies on available technology and enzyme innovation. The proposal was based on the development of a battery of microbiological, biochemical, and molecular biology techniques, with some innovative methodologies, such as (i) creation of an environmental DNA library containing only the coding sequences of the proteins; (ii) immobilization of holoenzymes on chemical supports; (iii) use of liqueurs obtained by auto-hydrolysis of agro-industrial residues; (iv) detoxification of sugarcane bagasse subjected to steam explosion, by laccases, to generate second-generation ethanol; (v) heterologous expression of fungi prospected in nature, facilitating cellulase and xylanase purification processes and increasing enzymatic expression.

As a general result, each group of researchers organized a collection of filamentous fungi isolated from nature or obtained by metagenomics. In addition, dozens of journal papers, book chapters, and a book were published (Polizeli & Rai 2013). A partial list of publications is as follows: bioprospecting (Almeida et al. 2017, Pasin et al. 2020); taxonomy (de Oliveira et al. 2020, Contato et al. 2021); improvement of bioprocesses (Pinheiro et al. 2020), purification and enzyme modeling (Michelin et al. 2014, Heinen et al. 2018); heterologous expression of holoenzymes (Damasio et al. 2012, Almeida et al. 2020); enzyme engineering (Furtado et al. 2015); and patents for formulating enzymatic cocktails with high potential for degrading agro-industrial residues, resulting in a high amount of readily fermentable sugars by ethanol-producing yeasts (Polizeli et al. 2021, Polizeli et al. 2021).

Future projects such as the one just described can offer sustainability to the Circular Economy in Brazil, in the sense of seeking new microorganisms, new enzymes, and innovative technologies to produce clean fuel, such as ethanol from sugarcane, its residues, or other available biomasses, which are currently polluting agents for the environment.

Metagenomic Surveys of Brazilian Biomes

Metagenomics is a powerful approach to study microorganisms present in any given environment and their potential to maintain and improve ecosystem health without the need of cultivation. In addition, function-based metagenomics has provided unprecedent access to the metabolic potential of microbes in nature, including the ones found in harsh and hard-to-access environments, opening new opportunities for the application of unique metagenome-derived enzymes and secondary metabolites (Bikel et al. 2015).

In the project "Prospecting microbial metagenome of mangrove sediments in the search for novel bioactive compounds" (grant 2011/50809-5), Valéria Maia de Oliveira and collaborators have investigated microbial communities in mangrove ecosystems of São Paulo State using a combination of whole-metagenome and metatranscriptome sequencing, functional metagenomics (metagenomic libraries) and sequence and function-driven screening assays (grant 2011/50809-5). Mangroves are coastal ecosystems of transition between terrestrial and marine environments, which represent a promising source for bioprospecting enzymes due to their unique characteristics, such as high microbial activity, fluctuations in oxic/anoxic conditions and high concentration of organic matter and salinity (Thatoi et al. 2013). Unfortunately, these ecosystems have been particularly contaminated in the last decades due to industrial activities and accidental oil spills (Peixoto et al. 2011, Van Lavieren et al. 2012).

The occurrence of genes encoding biotechnologically relevant hydrolases in polluted and unpolluted mangrove soil microbial communities has been evaluated (Jimenez et al. 2015, Ottoni et al. 2017, Soares et al. 2017, Alves et al. 2020). The main results revealed a high proportion related to epoxide hydrolases and haloalkane dehalogenases in polluted mangroves, suggesting selection and putative involvement in local degradation/detoxification of the pollutants. The low protein similarity (less than 60%) of many metagenomic sequences to already-described gene products indicated that they may encode hitherto undescribed enzymes, including epoxide hydrolases, haloalkane dehalogenases, xylanases and amylases, opening perspectives for both production in an expression host and genetic screening of metagenomes (Jimenez et al. 2015, Ottoni et al. 2017, Alves et al. 2020).

The genetic potential of the polluted mangrove microbiota to degrade and/or tolerate oil hydrocarbons and antibiotics, biocides and heavy metals has also been evaluated (de Sousa et al. 2017, Sousa et al. 2020, Cabral et al. 2016, Cabral et al. 2018, Cabral et al. 2019). A diversity and biogeographic analysis of aromatic-ring-hydroxylating dioxygenase (ARHD) genes revealed the genetic potential of the microbiota from the polluted mangrove sediments for aromatic hydrocarbon biodegradation, with local prevalence of benzene 1,2-dioxygenase, 3-phenylpropionate dioxygenase and naphthalene 1,2-dioxygenase, and a considerable evolutionary proximity among the dioxygenase families found in Antarctica and South America sites, in addition to high level of endemism in each continental region (de Sousa et al. 2017). Further, the activity-based search for aromatic hydrocarbon degradation genes in the metagenomic fosmid library derived from the polluted mangrove corroborated the previous findings and allowed the recovery of a plethora of genes corresponding to proteins and functional domains directly or indirectly involved in the aromatic compound metabolism (Sousa et al. 2020). Whole-metagenome sequencing of mangrove sediments revealed that genes and transcripts associated with resistance to toxic compounds and with aromatic compound degradation, particularly the meta and orthopathways, were more abundant in polluted mangroves, suggesting that many of the aromatic compounds are being aerobically degraded by the microbiota in these sites (Cabral et al. 2016, Cabral et al. 2018). Members of Gammaproteobacteria and Deltaproteobacteria classes displayed specific functions related to heavy metal resistance, potentially contributing to the detoxification of the polluted soil and playing a key role in the successful persistence of the microbial community in such sites (Cabral et al. 2016). Combined results provided strong evidence that the indigenous mangrove sediment microbiota developed essential mechanisms towards ecosystem remediation of petroleum hydrocarbon impact and that degradation of aromatic compounds plays an active role in the biological response to mangrove sediment pollution.

Additional efforts to explore the mangroves located along the coastline of the State of São Paulo were carried out by the research groups of I. S. Melo (2004/13910-6) and Fernando Dini Andreote (grant 2012/06245-2) by using a varied set of microbiological approaches, from culture-based analyses and bioprospecting (Dias et al. 2009, Soares et al. 2013) through fingerprinting analyses of microbial communities (Dias et al. 2011, Fasanella et al. 2012), metagenomics (Andreote et al. 2012) and metatranscriptomics (Cotta et al. 2019). Results allowed to gain knowledge on the microbial diversity present in this particular ecosystem, revealing the changes in the composition of the mangrove microbiomes due to contamination events associated to proximity with urban areas and also derived from oil spills (Andreote et al. 2012).

Microbiomes colonizing Amazonian niches, mainly soils, have been evaluated by Siu Mui Tsai and collaborators over the last 10 years (grants 2011/50914-3 and 2014/50320-4). These studies have characterized natural systems, and also compared their assemblages in forests (primary and secondary) with agricultural areas (crops and pastures). Rodrigues et al. (Rodrigues et al. 2013) have shown the role of pasture establishment in Amazonia, which results in the homogenization of the soil microbiome, limiting the occurrence of beta-diversity when compared to natural forests. The recovery of the microbiome functioning after the selection imposed by pasture was demonstrated later (Paula et al. 2014), where areas of secondary forests revealed a microbiome functioning capacity (as determined by microarray analyses) similar to that of primary forest, while pasture areas revealed a decrease of approximately 30% of the functional gene richness.

By analyzing carbon transformation (focused on methane production and transformation), Meyer et al. (Meyer et al. 2020) revealed the possibility to predict CH_4 fluxes through the description of microbiomes found in Amazonian soils. These authors compared primary forest with recovered areas and pastures, indicating the trigger of methane emission in pastures, while secondary forests showed recovery by reestablishing C cycling, as compared to natural areas. Using a similar sampling approach, Pedrinho et al. (Pedrinho et al. 2020) have shown the natural recovery of N cycling functions in soils after pasture abandonment in Amazonia. These observations corroborate the hypothesis that ecosystems organized in high levels of biodiversity are resilient to temporal changes, reinforcing the need for effective preservation of natural ecosystems and its residing microbiomes. Another more recent result is that Amazonian deforestation might result in the enrichment of antibiotic resistance genes in soils (Lemos et al. 2020).

The microbiome composition in the Amazoniarn Dark Earth (ADE) was also addressed by metagenomics analyses (Lemos et al. 2016). In addition, Brossi et al. (Brossi et al. 2014) have shown the high contents of *bph* genes in the ADE, relating the microbiome composition and functioning with the generation and maintenance of this particular soil system.

The phyllosphere of plants in the Atlantic rainforest has been addressed by Marli F. Fiore and collaborators and R. R. Rodrigues and co-workers, as part of two projects funded by the BIOTA/FAPESP Program (grants 1999/09635-0 and 2008/50824-1). These projects allowed the description of particular microbiome assemblages present in each plant species, even within a tropical forest (Lambais et al. 2006). More specifically, the cyanobacterial communities revealed to follow the same pattern, presenting different communities' assemblages in each plant species sampled (Rigonato et al. 2016). These results strongly connect microbial diversity with plant diversity, indicating the dependency of ecosystem preservation in order to proper guarantee the microbiome diversity.

In another project (grant 2011/50870-6), led by João Carlos Setubal and Aline Maria da Silva, the aim was the study of the composting microbiome found in the composting unit at the São Paulo Zoo, using metagenomics and metatranscriptomic techniques. Composting is carried out by complex microbial communities that in general include fungi and bacteria (Jurado et al. 2014). Composting microbiomes have enormous diversity of mesophilic and thermophilic microorganisms depending on the composting substrate, procedure, and surrounding conditions, and these microbial populations change as composting occurs (Antunes et al. 2016). Composting microbiota can be exploited for biomass-degrading microorganisms, but also for soil bioremediation and suppressiveness against plant diseases (Kolinko et al. 2018, Silva et al. 2019, Lutz et al. 2020, Palaniveloo et al. 2020). Setubal et al. have found that the Zoo composting process is overwhelmingly carried out by bacteria (Martins et al. 2013). They have presented detailed catalogs of the bacterial taxa that could be identified, using both 16S rRNA data as well as whole genome shotgun data (Antunes et al. 2016). Using the latter, they have been able to obtain 60 metagenome-assembled genomes (MAGs), more than 20 of which belong to completely novel species (Braga et al. 2021). These MAGs and the metatranscriptome data have in turn allowed the team to propose a time-dependent metabolic interaction model that gives a general view of the composting bacterial dynamic (Braga et al. 2021). These results are a contribution for the future engineering of efficient biomass-degrading thermophilic microbiomes.

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Conflicts of Interest

The authors declare that they do not have conflicts of interest related to the publication of this manuscript.

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